

FIGURE S1. Preparation of chMDMs from chicken whole blood. Chicken monocytes and macrophages were specifically recognised by mAb KUL01: PE and mouse-anti-chicken MHC II: FITC. (a) PBMCs isolated from chicken whole blood are gated within population P1. (b) PBMC-derived macrophages obtained from adherent cells after 48 h of incubation were gated in within P2. KUL01⁺/MHCII⁺ cells are shown in quadrant Q2 in both (a) and (b) accordingly by appropriate isotype control and compensation. Over 95% of adherent cells from PBMCs from chicken whole blood being regarded as chMDMs after 2 d of conversion and removal of non-adherent cells as they were KUL01⁺MHCII⁺. The dot-plots represent independent experiments of preparing PBMC-derived macrophages from individual batches of chicken whole blood.

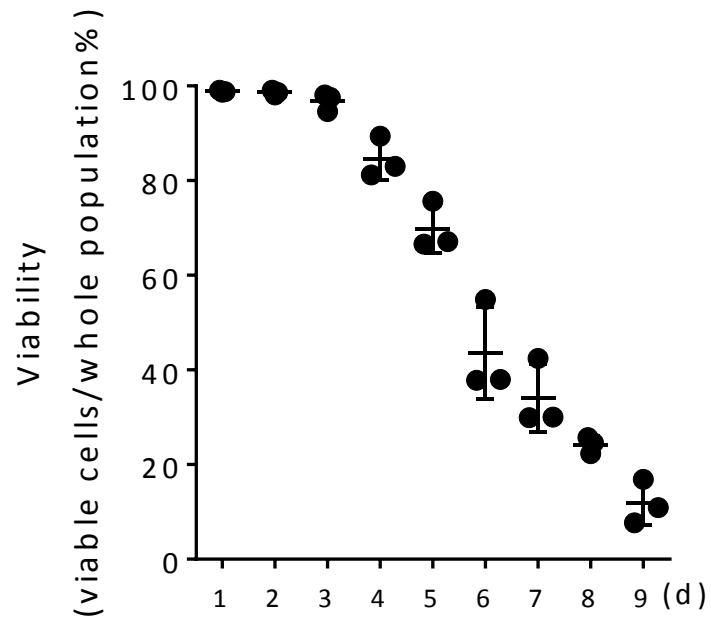


FIGURE S2. Viability of CD4⁺ T cells cultured *in vitro*. CD4⁺ T cells were stained with PI (20 µg/ml) and analysed during 9 d of culture *in vitro* to detect the percentage of viable cells within whole population of CD4⁺ T cells at each day post isolation. The results were shown as mean±SEM (n=3) of two independent experiments.

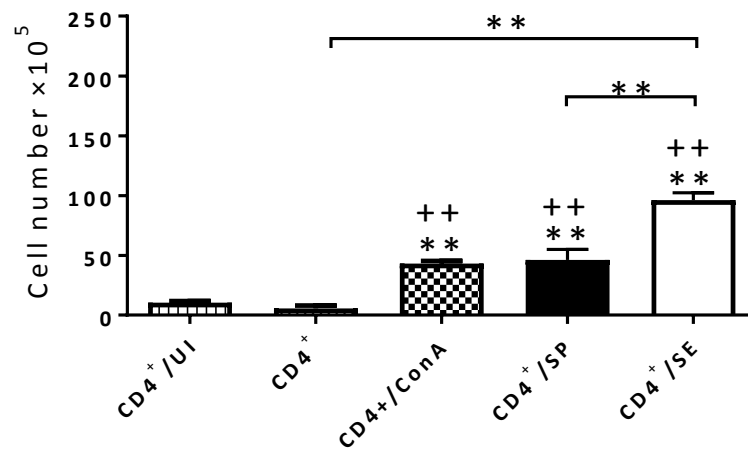


FIGURE S3. SP infection does not suppress proliferation of co-cultured CD4⁺ T cells sourced from unvaccinated birds after 5 d of co-culture. The experiment was done under the same condition and data was shown as described with in Fig. 6A.